# A Novel and Efficient Route towards α-GalNAc-Ser and α-GalNAc-Thr Building Blocks for Glycopeptide Synthesis

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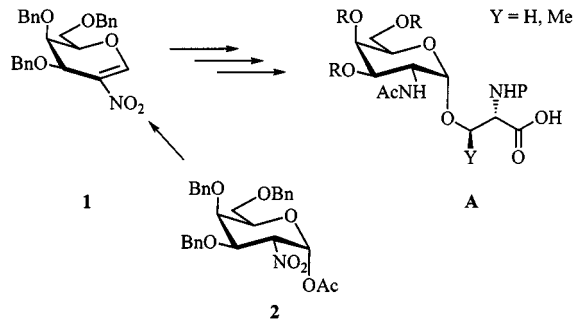
Michael addition of serine and threonine derivatives **4a**–**4c** to 3,4,6-tri-*O*-benzyl-2-nitro-D-galactal (**1**) afforded the corresponding 2-deoxy-2-nitro-α-D-galactopyranosides **5a**–**5c** in good yield and stereoselectivity. 2-deoxy-2-nitroglycosides **5a** and **5b** were reduced to the 2-acetamido compounds by platinized Raney nickel T4. Manipulation of the protecting groups afforded known *N*-Fmoc-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-L-serine (**8a**) and -threonine (**8b**), valuable building blocks for *O*-glycopeptide synthesis.

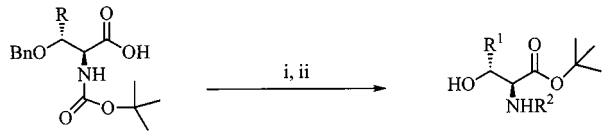
The α-glycosidic linkage between 2-acetamido-2-deoxy-D-galactopyranose and the side-chain hydroxy groups of L-serine or L-threonine is a common motif in numerous glycoproteins.[1] It is found in mucins, cell-membrane glycoproteins, blood-group determinants, immunoglobulins, anti-freeze glycoproteins and glycoprotein hormones.[1−3] Chemical synthesis of this 1,2-*cis*-glycosidic bond of 2-acetamido-2-deoxy-D-galactopyranosides proved to be difficult, since it necessitates a non-participating latent amino function at the C-2 atom of the glycosyl donor.[4] Lemieux et al.[5] addressed this problem using addition reactions to 2-nitrosoglycals and observed good α stereoselectivity. The reduction of the resulting 2-acetoximino-α-D-*lyxo*-hexopyranosides, however, afforded mixtures of epimeric *galacto* and *talo* products.[6] After the introduction of 2-azido-2-deoxyaldoses to glycoside synthesis by Paulsen et al.[7−9] and the azidonitration protocol by Lemieux,[10] derivatives of 2-azido-2-deoxygalactose remained the only possible glycosyl donors for the construction of the α-glycosidic bond of *N*-acetylgalactosamine to serine and threonine.[11−17] Recently, Michael-type addition to 3,4,6-tri-*O*-benzyl-2-nitro-D-galactal (**1**)[18] was shown to be a convenient glycosylation method for the synthesis of α-glycosides of galactosamine,[18] avoiding the often lengthy preparation of 2-azido-2-deoxygalactosyl donors. We present a new and efficient procedure based on this concept for the preparation of α-GalNAc-Ser and α-GalNAc-Thr building blocks of type **A** for glycopeptide synthesis (Scheme 1).

As glycosyl donor known 3,4,6-tri-*O*-benzyl-2-nitro-D-galactal (**1**) was used, which was prepared according to a slight modification of the reported procedure from 1-*O*-acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-2-nitro-α-D-galactopyranose (**2**).[18] Glycosylation was performed with serine and threonine acceptors carrying the protecting-group patterns *N*-fluoren-9-ylmethoxycarbonyl (Fmoc)/*O*-*tert*-butyl (*t*Bu)[19] and *N*-*tert*-butyloxycarbonyl (Boc)/*Ot*Bu. The




Scheme 1

required *N*-Boc/*Ot*Bu amino acids **4a** and **4b** were obtained from commercial Boc-serine/threonine-(*O*-benzyl)-OH (**3a** and **3b**) in two steps using *tert*-butyltrichloroacetimidate[20] and subsequent debenzylation (Scheme 2).




**3a**: R = H
**3b**: R = Me

**4a**: $R^1$ = H; $R^2$ = BOC
**4b**: $R^1$ = Me; $R^2$ = BOC
**4c**: $R^1$ = H; $R^2$ = Fmoc

Scheme 2. Reagents: i) *tert*-butyltrichloroacetimidate, $BF_3{\cdot}Et_2O$, $CH_2Cl_2$, cyclohexane; ii) $Pd(OH)_2/C$, $H_2$, EtOH

First, the glycosylation of Fmoc-L-Ser-*t*Bu (**4c**)[19,21] with **1** was established (Scheme 3). Since strong bases were reported to favour addition of the aglycon from the α side,[18] sterically hindered potassium *tert*-butoxide was chosen as base and found to be most efficient in catalytic quantities (0.1 equiv.) in combination with dry toluene as solvent. The reaction proceeds reasonably fast at room temperature and all of **1** is consumed after 3 h. As products the α-glycoside **5c** (83%) and the corresponding β-glycoside **5cβ** (14%) were isolated, showing a very good overall yield of glycosides and good α stereoselectivity. No loss of Fmoc protection was observed in the reaction medium. To ascertain that no racemization occurs during the base-catalysed glycosyl-

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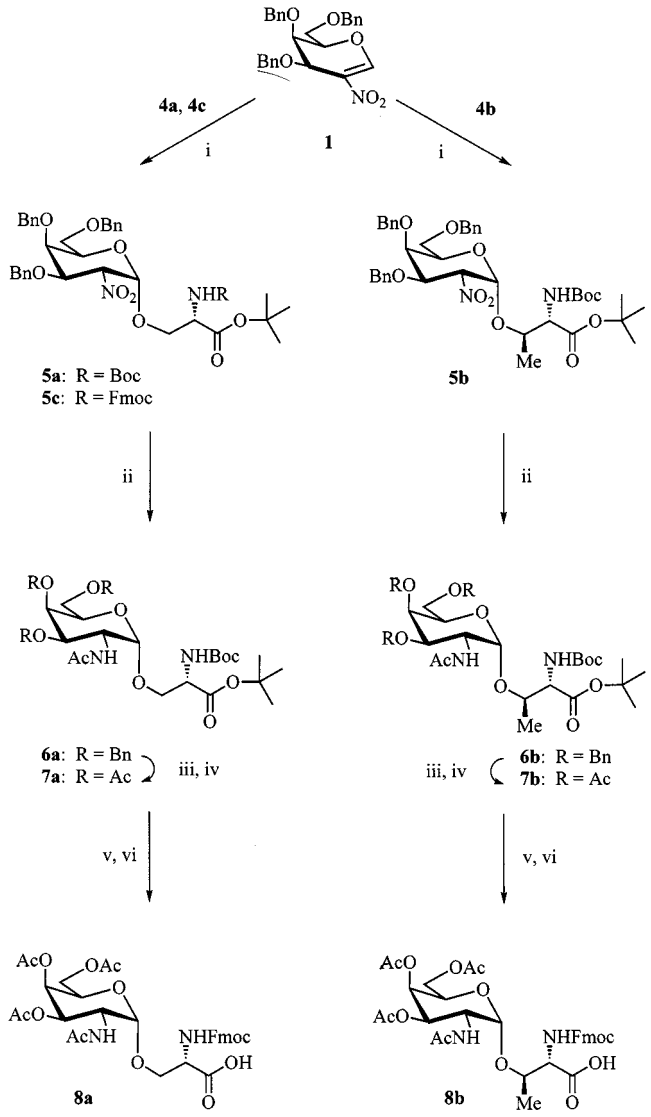
ation, the corresponding D-Ser derivative of **5c**, D-**5c**, was synthesized. TLC analysis (toluene/ethyl acetate, 9:1) clearly separated D- and L-serine glycosides and confirmed that no D-serine glycoside is formed during the glycosylation of **4c**. For Fmoc protection of the amino function does not resist hydrogenolytic conditions, the protecting-group pattern Boc/*t*Bu was then envisaged to extend the synthesis to known 2-acetamido-3,4,6-tri-*O*-acetylgalactose derivatives. Glycosylation with Boc-L-Ser-*t*Bu (**4a**) works equally efficiently and affords **5a** (80%) and **5aβ** (13%). The addition of the secondary hydroxy group of threonine derivative **4b** to 2-nitrogalactal **1** proceeds highly selectively from the α side yielding glycoside **5b** in excellent yield (98% based on consumed **1**) with some glycosyl donor (20%) remaining unchanged.




Scheme 3. Reagents: i) *t*BuOK, toluene; ii) Ra-Ni T4-Pt, $H_2$ EtOH; iii)Pd/C, $H_2$, MeOH, AcOH; iv) $Ac_2O$, pyridine; v) TFA, $CH_2Cl_2$; vi) Fmoc-ON-Su, $NaHCO_3$, MeCN, $H_2O$

For the reduction of the nitro group the system platinized Ra-Ni T4/$H_2$[22] was found to work very efficiently under convenient experimental conditions. 2-Acetamido derivatives **6a** (89%) and **6b** (84%, Scheme 3) were thus obtained after reduction at ambient pressure and temperature and subsequent *N*-acetylation. Exchange of *O*-benzyl protection on the carbohydrate moiety by *O*-acetyl groups afforded **7a** and **7b**. Cleavage of Boc and *t*Bu protection with the help of trifluoroacetic acid and attachment of the Fmoc group to the amino function gave known building blocks **8a**[19][23] and **8b**[19][23] in good overall yield. The importance of these building blocks for *O*-glycopeptide synthesis is well documented.[4]

## Experimental Section

**General:** Dry solvents were purchased from Kanto Chemicals Inc. − Column chromatography: Silica gel 60 N 40−100μm (Kanto Chemicals) and silica gel for flash chromatography 40 μm (J. T. Baker). − Analytical TLC: HPTLC plates, silica gel 60 $F_{254}$ (Merck), and TLC plastic sheets, silica gel 60 $F_{254}$ (Merck); detection with UV light (254 nm) and with 5% $(NH_4)_2MoO_4$, 0.1% $Ce(SO_4)_2$ in 10% $H_2SO_4$ and heating to 160°C. − Melting points: Büchi 510 melting-point apparatus, values uncorrected. − Optical rotations: JASCO DIP 370 polarimeter or Perkin−Elmer polarimeter 241 MC in 1-dm cells at 22°C. − IR spectra: Shimadzu FT-IR 8100 M, solutions in $CHCl_3$ on NaCl cells. − $^1$H-NMR spectra: Bruker AC 250 (250 MHz) Cryospec, JEOL EX 270 MHz spectrometer, JEOL EX 400 MHz spectrometer, JEOL EX 500-MHz spectrometer or Bruker DRX 600 (600 MHz); tetramethylsilane as internal standard. − $^{13}$C-NMR spectra: JEOL EX 270-MHz spectrometer (68 MHz); tetramethylsilane as internal standard. − FAB mass spectra: JEOL HX 110 from an *m*-nitrobenzyl alcohol matrix with NaI as additive. − MALDI mass spectra: Kratos Analytical Kompact Maldi from 2,5-dihydroxybenzoic acid.

**3,4,6-Tri-*O*-benzyl-2-nitro-D-galactal (1):** 1-*O*-Acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-2-nitro-α-D-galactopyranose (**2**)[18] (2.0 g, 3.96 mmol) was dissolved in $CH_2Cl_2$ (5 mL) and slowly added to an ice-cold, stirred solution of $NEt_3$ (0.66 mL, 4.75 mmol) in $CH_2Cl_2$ (5 mL). After complete addition, the cooling bath was removed and stirring continued for 20 min. The organic phase was washed with 2 M HCl solution and dried with $Na_2SO_4$. Removal of the volatiles and column-chromatographic purification (toluene/ethyl acetate, 98:2) of the residue furnished **1** (1.45 g, 79%). Analytical data are in accordance with the literature.[18]

***N*-(*tert*-Butyloxycarbonyl)-L-serine *tert*-Butyl Ester (4a):** A solution of *tert*-butyltrichloroacetimidate (1.48 g, 6.77 mmol) in cyclohexane (6.8 mL) was added to a stirred solution of commercial **3a** (1.0 g, 3.39 mmol) in $CH_2Cl_2$ (3.4 mL) followed by boron trifluoride−diethyl ether (68 μL, 0.55 mmol). Stirring was continued for 14 h before solid $NaHCO_3$ was used to neutralize the acid. Evaporation of the solvents and purification on a short silica-gel column (toluene/ethyl acetate, 9:1) afforded the intermediate *N*-Boc-L-serine-(*O*Bn) *tert*-butyl ester (1.14 g, 96%). This material was dissolved in ethanol/acetic acid (5:1, 12 mL) and stirred together with $Pd(OH)_2$/C (0.11 g, 20% Pd) under $H_2$ for 36 h. Filtration through Celite and evaporation of all volatiles gave **4a** (0.80 g, 90%), which crystallized upon standing, m.p. 80°C. − TLC (toluene/ethyl acetate, 4:1): $R_f$ = 0.33. − $[\alpha]_D^{22}$ = −22.5 ($c$ = 1.8, ethanol). − $^1$H NMR (270 MHz, $CDCl_3$): δ = 5.40 (br. s, 1 H, NH), 4.25 (br. s, 1 H, α-CH), 3.90 (br. s, 2 H, $CH_2$), 2.35 (br. s, 1 H, OH), 1.49 (s, 9 H, $C_4H_9$), 1.46 (s, 9 H, $C_4H_9$). − MS (FAB): calcd. 261.16 + 22.99 (Na) = 284.15; found 284.16 $(M + Na)^+$. − Ref.[24] m.p. 76−78°C, $[\alpha]_D^{22}$ = −20.0 ($c$ = 1.8, ethanol).

***N*-(*tert*-Butyloxycarbonyl)-L-threonine *tert*-Butyl Ester (4b):** Commercial **3b** (1.0 g, 3.23 mmol) was treated as described for **4a** to afford intermediate *N*-Boc-threonine-(*O*Bn) *tert*-butyl ester (1.10 g, 93%). Hydrogenation as for **4a** gave **4b** (0.82 g, 92%), which crystallized upon standing, m.p. 70°C. − TLC (toluene/ethyl acetate, 4:1): $R_f$ = 0.38. − $[\alpha]_D^{22}$ = −23.4 (*c* = 1.0, ethanol). − $^1$H NMR (270 MHz, $CDCl_3$): δ = 5.24 (br. d, 1 H, NH), 4.23 (br. d, 1 H, β-CH), 4.13 (br. d, 1 H, α-CH), 1.97 (br. s, 1 H, OH), 1.49 (s, 9 H, $C_4H_9$), 1.46 (s, 9 H, $C_4H_9$), 1.24 (d, 3 H, $CH_3$). − $C_{13}H_{25}NO_5$ (275.2): calcd. C 56.71, H 9.15, N 5.09; found C 56.35, H 9.16, N 5.02. − MS (FAB): calcd. 275.17 + 22.99 (Na) = 298.16; found 298.14 $(M + Na)^+$.

***O*-(3,4,6-Tri-*O*-benzyl-2-deoxy-2-nitro-α-D-galactopyranosyl)-*N*-(*tert*-butyloxycarbonyl)-L-serine *tert*-Butyl Ester (5a): 1** (0.25 g, 0.54 mmol) and **4c** (0.15 g, 0.57 mmol) were dried under high vacuum and dissolved in dry toluene (25 mL) under argon. Freshly activated molecular sieve (3 Å, 0.3 g) was introduced and the mixture stirred for 1 h. Then 1 M potassium *tert*-butoxide solution in THF (53 μL, 0.05 mmol) was added and stirring continued for 75 min. Acetic acid (50 mL) was used to acidify the reaction mixture, the molecular sieve was filtered off and all solvents were removed. The residue was purified by column chromatography (toluene/ethyl acetate, 9:1) to furnish **5a** (0.31 g, 80%) and the corresponding β-glycoside **5aβ** (0.05 g, 13%). − **5a**: TLC (toluene/ethyl acetate, 9:1): $R_f$ = 0.48. − $[\alpha]_D^{22}$ = 67.0 (*c* = 1.0, chloroform). − IR: $\tilde{\nu}$ = 1561cm$^{-1}$ ($NO_2$). − $^1$H NMR (400 MHz, $CDCl_3$): δ = 7.37−7.19 (m, 15 H, arom. H), 5.44 (d, $J_{NH,\alpha\text{-}CH}$ = 7.81 Hz, 1 H, NH), 5.27 (d, $J_{H1,H2}$ = 3.91, 1 H, $H_1$), 4.96 (dd, $J_{H2,H1}$ = 4.39, $J_{H2,H3}$ = 10.73, 1 H, $H_2$), 4.82 (d, $J_{gem\ H}$ = 10.73, 1 H, benzyl. H), 4.72 (s, 2 H, benzyl. H), 4.53−4.40 (m, 3 H, benzyl. H), 4.38 (dd, $J_{H3,H2}$ = 10.73, $J_{H3,H4}$ = 3.90, 1 H, $H_3$), 4.31 (br. d, $J_{\alpha\text{-}CH,NH}$ = 7.81, 1 H, α-CH), 4.04−3.99 (m, 2 H, $H_4$, $H_5$), 3.88 (d, $J_{\beta\text{-}CH2,\alpha\text{-}CH}$ = 2.93, 2 H, β-$CH_2$), 3.58−3.55 (m, 2 H, $H_6$, $H_{6'}$), 1.46 (s, 9 H, $C_4H_9$), 1.44 (s, 9 H, $C_4H_9$). − $^{13}$C NMR (68 MHz, $CDCl_3$): δ = 168.65 (1 C, COOR), 155.38 (1 C, CONHR), 137.87, 137.62, 137.23 (3 C, subst. arom. C), 128.47−127.83 (15 C, arom. C), 96.71 (1 C, $C_1$), 83.94 (1 C, $C_2$), 82.79 (1 C, *tert*-Bu ester quat. C), 79.93 (1 C, Boc quat. C), 75.10 (1 C, benzyl. C), 74.93 (1 C, $C_3$), 73.51 (1 C, benzyl. C), 72.98 (1 C, $C_4$), 72.90 (1 C, benzyl. C), 69.71 (2 C, $C_5$, β-C), 67.94 (1 C, $C_6$), 54.24 (1 C, α-C), 28.29 (3 C, Boc prim. C), 27.86 (3 C, *tert*-Bu ester prim. C). − $C_{39}H_{50}N_2O_{11}$ (722.3): calcd. C 64.80, H 6.97, N 3.88; found C 64.91, H 6.97, N 3.76. − MS (FAB): calcd. 722.34 + 22.99 (Na) = 745.3; found 745.4 $(M + Na)^+$. − **5aβ**: TLC (toluene/ethyl acetate, 9:1): $R_f$ = 0.30. − $^1$H NMR (270 MHz, $CDCl_3$): δ = 5.23 (d, $J_{H1,H2}$ = 7.42, 1 H, $H_1$).

***O*-(3,4,6-Tri-*O*-benzyl-2-deoxy-2-nitro-α-D-galactopyranosyl)-*N*-(*tert*-butyloxycarbonyl)-L-threonine *tert*-Butyl Ester (5b): 1** (46 mg, 0.10 mmol) and **4b** (30 mg, 0.11 mmol) were dried under high vacuum and dissolved in dry toluene (5 mL) under argon. Freshly activated molecular sieve (3 Å, 0.1 g) was introduced and the mixture stirred for 1 h. Then 1 M potassium *tert*-butoxide solution in THF (10 μL, 0.05 mmol) was added and stirring continued for 4 h. Acetic acid (10 μL) was used to acidify the reaction mixture, the molecular sieve was filtered off and all solvents were removed. The residue was purified by column chromatography (toluene/ethyl acetate, 9:1) to furnish recovered **1** (9 mg, 20%) and **5b** (58 mg, 98% based on consumed **1**). No corresponding β-glycoside could be detected. − TLC (toluene/ethyl acetate, 9:1): $R_f$ = 0.45. − $[\alpha]_D^{22}$ = 71.2 (*c* = 1.0, chloroform). − IR: $\tilde{\nu}$ = 1561cm$^{-1}$ ($NO_2$). − $^1$H NMR (500 MHz, $CDCl_3$): δ = 7.40−7.16 (m, 15 H, arom. H), 5.39 (d, $J_{H1,H2}$ = 4.10, 1 H, $H_1$), 5.02 (d, $J_{NH,\alpha\text{-}CH}$ = 10.00 Hz, 1 H, NH), 4.96 (dd, $J_{H2,H1}$ = 4.10, $J_{H2,H3}$ = 10.77, 1 H, $H_2$), 4.83 (d, $J_{gem\ H}$ = 11.28, 1 H, benzyl. H), 4.72 (s, 2 H, benzyl. H), 4.50−4.40 (m, 4 H, 3 benzyl. H, $H_3$), 4.32 (br. dd, $J_{\beta\text{-}CH,\gamma\text{-}CH3}$ = 6.15, 1 H, β-CH), 4.10 (d, $J_{\alpha\text{-}CH,NH}$ = 10.00, 1 H, α-CH), 4.05−4.03 (m, 2 H, $H_4$, $H_5$), 3.58−3.51 (m, 2 H, $H_6$, $H_{6'}$), 1.47 (s, 9 H, $C_4H_9$), 1.46 (s, 9 H, $C_4H_9$), 1.28 (d, $J_{\gamma\text{-}CH3,\beta\text{-}CH}$ = 6.41, 3 H, γ-$CH_3$). − $^{13}$C NMR (68 MHz, $CDCl_3$): δ = 169.19 (1 C, COOR), 156.19 (1 C, CONHR), 137.86, 137.64, 137.25 (3 C, subst. arom. C), 129.02−127.72 (15 C, arom. C), 96.43 (1 C, $C_1$), 84.26 (1 C, $C_2$), 82.67 (1 C, *tert*-Bu ester quat. C), 79.90 (1 C, Boc quat. C), 75.76 (1 C, β-C), 75.15 (1 C, benzyl. C), 75.04 (1 C, $C_3$), 73.55 (1 C, benzyl. C), 73.01 (2 C, $C_4$, benzyl. C), 69.94 (1 C, $C_5$), 68.19 (1 C, $C_6$), 58.57 (1 C, α-C), 28.34 (3 C, Boc prim. C), 27.95 (3 C, *tert*-Bu ester prim. C), 18.67 (1 C, γ-C). − $C_{40}H_{52}N_2O_{11}$ (736.4): calcd. C 65.20, H 7.11, N 3.80; found C 65.27, H 7.13, N 3.55. − MS (FAB): calcd. 736.36 + 22.99 (Na) = 759.4; found 759.2 $(M + Na)^+$.

***O*-(3,4,6-Tri-*O*-benzyl-2-deoxy-2-nitro-α-D-galactopyranosyl)-*N*-(fluorenylmethoxycarbonyl)-L-serine *tert*-Butyl Ester (5c): 1** (46 mg, 0.10 mmol) and **4c**[21] (42 mg, 0.11 mmol) were dried under high vacuum and dissolved in dry toluene (5 mL) under argon. Freshly activated molecular sieve (3 Å, 0.1 g) was introduced and the mixture stirred for 1 h. Then 1 M potassium *tert*-butoxide solution in THF (10 μL, 0.01 mmol) was added and stirring continued for 3 h. Acetic acid (10 μL) was used to acidify the reaction mixture, the molecular sieve was filtered off and all solvents were removed. The residue was purified by column chromatography (toluene/ethyl acetate, 9:1) to furnish **5c** (70 mg, 83%) and the corresponding β-glycoside **5cβ** (12 mg, 14%). − **5c**: TLC (toluene/ethyl acetate, 9:1): $R_f$ = 0.48. − $[\alpha]_D^{22}$ = 58.5 (*c* = 1.0, chloroform). − IR: $\tilde{\nu}$ = 1561 cm$^{-1}$ ($NO_2$). − $^1$H NMR (500 MHz, $CDCl_3$): δ = 7.76−7.19 (m, 23 H, arom. H), 5.89 (d, $J_{NH,\alpha\text{-}CH}$ = 7.62, 1 H, NH), 5.27 (d, $J_{H1,H2}$ = 4.10, 1 H, $H_1$), 4.98 (dd, $J_{H2,H1}$ = 4.10, $J_{H2,H3}$ = 10.55, 1 H, $H_2$), 4.82 (d, $J_{gem\ H}$ = 11.43, 1 H, benzyl. H), 4.75−4.70 (m, 2 H, benzyl. H), 4.46−4.31 (m, 7 H, 3 benzyl. H, $H_3$, 2 Fmoc-$CH_2$, α-CH), 4.21−4.19 (m, 1 H, Fmoc-CH), 4.01−3.92 (m, 4 H, $H_4$, $H_5$, β-$CH_2$), 3.55−3.52 (m, 1 H, $H_6$), 3.48−3.44 (m, 1 H, $H_{6'}$), 1.48 (s, 9 H, $C_4H_9$). − $^{13}$C NMR (68 MHz, $CDCl_3$): δ = 168.36 (1 C, COOR), 143.85−119.96 (30 C, arom. C), 97.00 (1 C, $C_1$), 83.99 (1 C, $C_2$), 83.07 (1 C, *tert*-Bu ester quat. C), 75.04 (2 C, benzyl. C, $C_3$), 73.42, 73.07, 72.99 (3 C, 2 benzyl. C, $C_4$), 70.08, 69.81 (2 C, $C_5$, β-C), 68.32 (1 C, $C_6$), 67.03 (1 C, Fmoc-$CH_2$), 54.72 (1 C, α-C), 47.14 (1 C, Fmoc-CH), 27.89 (3 C, *tert*-Bu ester prim. C). − $C_{49}H_{52}N_2O_{11}$ (844.4): calcd. C 69.65, H 6.20, N 3.32; found C 69.46, H 6.21, N 3.21. − MS (FAB): calcd. 844.4 + 22.99 (Na) = 867.4; found 867.4 $(M + Na)^+$. − **5cβ**: TLC (toluene/ethyl acetate 9:1): $R_f$ = 0.33. −$^1$H NMR (270 MHz, $CDCl_3$): δ = 5.60 (d, $J_{H1,H2}$ = 7.92, 1 H, $H_1$).

***O*-(3,4,6-Tri-*O*-benzyl-2-deoxy-2-nitro-α-D-galactopyranosyl)-*N*-(fluorenylmethoxycarbonyl)-D-serine *tert*-Butyl Ester (D-5c):** D-**5c** was synthesized exactly as **5c** starting from Fmoc-D-Ser-*t*Bu (D-4c).[21] − TLC (toluene/ethyl acetate, 9:1): $R_f$ = 0.41. − $^1$H NMR (270 MHz, $CDCl_3$): δ = 7.77−7.15 (m, 23 H, arom. H), 5.52 (d, $J_{NH,\alpha\text{-}CH}$ = 8.44, 1 H, NH), 5.31 (d, $J_{H1,H2}$ = 3.96, 1 H, $H_1$), 5.01 (dd, $J_{H2,H1}$ = 4.12, $J_{H2,H3}$ = 10.72, 1 H, $H_2$), 4.83 (d, $J_{gem\ H}$ = 11.21, 1 H, benzyl. H), 4.71 (s, 2 H, benzyl. H), 4.53−4.22 (m, 9 H), 4.15−4.12 (m, 1 H), 3.97−3.93 (m, 2 H), 3.66−3.63 (m, 1 H), 3.53 (d, 2 H), 1.48 (s, 9 H, $C_4H_9$).

***O*-(2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-*N*-(*tert*-butyloxycarbonyl)-L-serine *tert*-Butyl Ester (6a): 5a** (0.40 g, 0.55 mmol) was dissolved in ethanol (7 mL) and transferred to a hydrogenation vessel. Platinized Raney nickel T4 catalyst was freshly prepared as described in ref.[22] and the material obtained from 2 g of Raney nickel/aluminium alloy was suspended in ethanol

(15 mL). From a homogeneous suspension of this catalyst 7 mL was added to the reaction vessel and the suspension shaken under $H_2$ for 48 h at ambient temperature and pressure. The catalyst was filtered off and the solvent evaporated. The residue was dissolved in pyridine/acetic anhydride (2:1, 9 mL) and stirred for 3 h. Removal of the volatiles and column-chromatographic purification (toluene/acetone, 3:1) gave **6a** (0.36g, 89%). − TLC (toluene/acetone, 3:1): $R_f$ = 0.54. − $[\alpha]_D^{22}$ = 77.5 ($c$ = 2.0, chloroform). − $^1$H NMR (270 MHz, $CDCl_3$): δ = 7.40−7.16 (m, 15 H, arom. H), 5.32 (br. d, 1 H, NH), 4.94 (d, $J_{gem\ H}$ = 11.54, 1 H, benzyl. H), 4.80 (d, $J_{H1,H2}$ = 3.63, 1 H, $H_1$), 4.73−4.62 (m, 2 H, $H_2$, benzyl. H), 4.58−4.39 (m, 4 H, benzyl. H), 4.32 (br. s, 1 H, α-CH), 4.00 (s, 1 H, $H_4$), 3.89−3.74 (m, 3 H, $H_5$, β-$CH_2$), 3.64−2.51 (m, 3 H, $H_3$, $H_6$, $H_{6'}$), 1.88 (s, 3 H, $CH_3CO$), 1.45 (s, 9 H, $C_4H_9$), 1.42 (s, 9 H, $C_4H_9$). − MS (FAB): calcd. 734.88 + 22.99 (Na) = 757.87; found 757.4 (M + Na)$^+$.

***O*-(2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-*N*-(*tert*-butyloxycarbonyl)-L-threonine *tert*-Butyl Ester (6b): 5b** (0.62 g, 0.85 mmol) was dissolved in ethanol (10 mL) and transferred to a hydrogenation vessel. The platinized Raney nickel T4 suspension (8 mL, see preparation of **6a**) was added to the reaction vessel and the mixture shaken under $H_2$ for 12 h at ambient temperature and pressure. The catalyst was filtered off and the solvent evaporated. The residue was dissolved in pyridine/acetic anhydride (2:1, 6 mL) and stirred for 3 h. Removal of the volatiles and column-chromatographic purification (toluene/ethyl acetate, 6:1−4:1) gave **6b** (0.53g, 84%). − TLC (toluene/acetone, 3:1): $R_f$ = 0.48. − $[\alpha]_D^{22}$ = 78.0 ($c$ = 1.0, chloroform). − $^1$H NMR (600 MHz, $CDCl_3$): δ = 7.37−7.24 (m, 15 H, arom H), 5.69 (s, 1 H, NH), 4.99−4.97 (m, 2 H, NH, benzyl. H), 4.79−4.72 (m, $J_{H1,H2}$ = 3.84, 3 H, $H_1$, $H_2$, benzyl. H), 4.57−4.40 (m, 4 H, benzyl. H), 4.12 (d, $J_{α\text{-}CH,β\text{-}CH}$ = 9.13, 1 H, α-CH),4.09−4.06 (m, 1 H, β-CH), 3.98 (s, 1 H, $H_4$), 3.93 (m, 1 H, $H_5$), 3.59−3.52 (m, 3 H, $H_3$, $H_6$, $H_{6'}$), 1.99 (s, 3 H, $CH_3CO$), 1.47 (s, 9 H, $C_4H_9$), 1.43 (s, 9 H, $C_4H_9$), 1.27 (d, $J_{γ\text{-}CH3,βCH}$ = 5.82, 3 H, γ-$CH_3$). − MS (MALDI): calcd. 749 + 23 (Na) = 772; found 773 (M + Na)$^+$.

***O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-*N*-(*tert*-butyloxycarbonyl)-L-serine *tert*-Butyl Ester (7a): 6a** (0.10 g, 0.14 mmol) was dissolved in methanol/acetic acid (1:1, 4 mL) and Pd/C (0.05 g, 10% Pd) was suspended in the solution. This mixture was stirred for 3 h under $H_2$. After complete disappearance of the starting material (TLC: $CHCl_3/CH_3OH$, 9:1), the catalyst was filtered off and all solvents were removed. The residue was treated with pyridine/acetic anhydride (2:1, 6 mL) and stirred at room temperature for 12 h. All volatiles were evaporated and the product purified by column chromatography (toluene/ethyl acetate, 1:1) to give **7a** (75 mg, 93%). − TLC (toluene/ethyl acetate, 1:1): $R_f$ = 0.19. − $[\alpha]_D^{22}$ = 65.5 ($c$ = 2.8, chloroform). − $^1$H NMR (600 MHz, $CDCl_3$): δ = 5.71 (br. d, $J_{NH,CH2}$ = 8.22, 1 H, AcNH), 5.38 (br. d, $J_{H4,H3}$ = 3.28, 2 H, $H_4$, BocNH), 5.11 (dd, $J_{H3,H2}$ = 11.30, $J_{H3,H4}$ = 3.28, 1 H, $H_3$), 4.84 (d, $J_{H1,H2}$ = 3.60, 1 H, $H_1$), 4.60 (ddd, $J_{H2,H1}$ = 3.60, $J_{H2,H3}$ = 11.10, $J_{H2,NH}$ = 9.90, 1 H, $H_2$), 4.35 (br. s, 1 H, $H_5$), 4.16−4.06 (m, $J_{α\text{-}CH,β\text{-}CH}$ = 6.12, $J_{α\text{-}CH,β'\text{-}CH}$ = 3.80, $J_{β\text{-}CH,β'\text{-}CH}$ = 10.09, 2 H, α-CH, β-CH), 4.08−4.06 (m, $J_{β'\text{-}CH,α\text{-}CH}$ = 3.99, $J_{β'\text{-}CH,β\text{-}CH}$ = 10.08, 1 H, β′-CH), 3.93 (dd, $J_{H6,H5}$ = 3.35, $J_{H6,H6'}$ = 10.51, 1 H, $H_6$), 3.83 (dd, $J_{H6',H5}$ = 3.17, $J_{H6',H6}$ = 10.51, 1 H, $H_{6'}$), 2.51, 2.06, 2.00, 1.95 (4 s, 12 H, $CH_3CO$), 1.48, 1.47 (2 s, 18 H, 2 $C_4H_9$). − MS (MALDI): calcd. 591 + 23 = 614; found 615 (M + Na)$^+$.

***O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-*N*-(*tert*-butyloxycarbonyl)-L-threonine *tert*-Butyl Ester (7b): 6b** (0.10 g, 0.13 mmol) was treated as described for the preparation of **7a** to give **7b** (78 mg, 96%). − TLC (toluene/ethyl acetate, 1:1): $R_f$ = 0.17. − $[\alpha]_D^{22}$ = 83.1 ($c$ = 1.0, chloroform). − $^1$H NMR (600 MHz, $CDCl_3$): δ = 5.99 (d, $J_{NH,CH2}$ = 9.96, 1 H, AcNH), 5.38 (d, $J_{H4,H3}$ = 3.36, 1 H, $H_4$), 5.20 (d, $J_{NH,α\text{-}CH}$ = 9.4, 1 H, BocNH), 5.08 (dd, $J_{H3,H2}$ = 11.32, $J_{H3,H4}$ = 3.21, 1 H, $H_3$), 4.87 (d, $J_{H1,H2}$ = 3.65, 1 H, $H_1$), 4.60 (ddd, $J_{H2,H1}$ = 3.69, $J_{H2,H3}$ = 10.76, $J_{H2,NH}$ = 10.25, 1 H, $H_2$), 4.23 (m, 1 H, $H_5$), 4.20−4.16 (m, $J_{α\text{-}CH,NH}$ = 9.7, $J_{β\text{-}CH,γ\text{-}CH}$ = 6.4, 2 H, α-CH, β-CH), 4.10−4.06 (m, 2 H, $H_6$, $H_{6'}$), 2.16, 2.04, 2.01, 2.00 (4 s, 12 H, $CH_3CO$), 1.49, 1.46 (2 s, 18 H, 2 $C_4H_9$), 1.33 (d, $J_{γ\text{-}CH3,β\text{-}CH}$ = 6.08, 3 H, γ-$CH_3$). − MS (MALDI): calcd. 605 + 23 (Na) = 628; found 629 (M + Na)$^+$.

***O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-*N*-(fluorenylmethoxycarbonyl)-L-serine (8a): 7a** (10 mg, 0.02 mmol) was dissolved in a mixture of trifluoroacetic acid and $CH_2Cl_2$ (2 mL, 1:1) and the solution stirred at room temperature for 12 h. Then all solvents were evaporated. The residue was redissolved together with Fmoc-ONSu (10 mg, 0.03 mmol) and $NaHCO_3$ (25 mg, 0.30 mmol) in acetonitrile/water (1:1, 4 mL) and the mixture stirred for 12 h. The solution was acidified with 2 M HCl solution (5 mL) and the aqueous layer extracted with $CH_2Cl_2$ (3 × 5 mL). The combined organic extracts were dried with $Na_2SO_4$ and the volatiles evaporated. Column-chromatographic separation (toluene/ethanol/acetic acid, 14:1:1) of the residue afforded **8a** (10 mg, 90%). − TLC (chloroform/methanol, 4:1): $R_f$ = 0.45. − $[\alpha]_D^{22}$ = 88.0 ($c$ = 0.3, chloroform); ref.[19] $[\alpha]_D^{20}$ = 89.9 ($c$ = 1.0, chloroform), ref.[23] $[\alpha]_D^{20}$ = 87.5 ($c$ = 2.0, chloroform).

***O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-*N*-(fluorenylmethoxycarbonyl)-L-threonine (8b): 7b** (10 mg, 0.02 mmol) was treated as described for the preparation of **8a**. Column-chromatographic separation (toluene/ethanol/acetic acid, 14:1:1) of the residue afforded **8b** (10 mg, 88%). − TLC (chloroform/methanol, 4:1): $R_f$ = 0.55. − $[\alpha]_D^{22}$ = 61.5 ($c$ = 0.2, chloroform); ref.[19] $[\alpha]_D^{20}$ = 65.0 ($c$ = 1.45, chloroform), ref.[23] $[\alpha]_D^{20}$ = 59.0 ($c$ = 0.50, chloroform).

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